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24th.June 1954.

Professor J.Lederberg,
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Dear Josh,

Thanks for your air letter of 16 May, and my apologies for not answering it sooner, (mainly because I have not much to report on the "abortives"). Thanks for details on technique, which are much what I thought; one difference with my conditions is that the only way I seem able to avoid losing whole experiments by failure to grow of all isolated "initials" is by using log-phase recipient cells; of course this makes it theoretically unlikely that I shall catch the real initials. However, I have a number of further pedigrees, all of which are compatible with the hypothesis I put forward in my last letter; the significant ones are some half-dozen in which I am sure an E cell was isolated and split up. In several experiments I was able to make a tentative diagnosis that a particular cell, or one of two, was the gene-bearer, and so concentrate on their progeny, and in at least two cases this diagnosis was confirmed by the behaviour of the progeny. The pedigrees are a bit too complicated to try and get into a letter, I will try and extract the essential bits of them later in diagram form. One of these pedigrees requires that the sib of the gene-bearer shall receive ten "gene-products" when the parent cell divides, and I see your data require eleven in one case. I feel fairly sure that the "gene-product" concerned is the flagellum itself (or whatever it springs from) though I still have no direct evidence on this. I think this (i) partly because it seems reasonable à priori and it explains the apparent absence of phenotypic lag in the real "semi-clones" (how do you like "mono-clone" for a clone of the type to be expected from a T cell with 1 gene product ?), and (ii) because of differences of phenotype which I think I can detect between cells which, on the hypothesis, are mono-flagellate and multi-flagellate respectively. This difference is that, for cells of the same growth phase, a multi-flagellate cell is less likely to get stuck to glass or oil than a mono-flagellate cell,

and shows less "nutation" of long axis about axis of travel; I am not sure if there is a difference in velocity. Also I had one cell, which from its pedigree must have been a T cell and on present hypothesis a mono-flagellate cell which showed a kind of motion I have never seen before. The cell was a diplo-bacillus travelling round the edge of a crowded droplet, i.e. "stationary phase" population; it was proceeding steadily along sideways, i.e. with its long axis perpendicular to the direction it was travelling in. Now and again it left the edge of the droplet, and in the deeper regions it continued to move broadside foremost, but there was now a slow rotation of the rod about an approximately central axis parallel to the direction of travel. This kind of motion fits in I think with what one would get from a single flagellum attached at mid-point (as to hydro-dynamic resistance) and exerting a thrust along its axis together with a torque about it, which is what a screw-like motion of flagellum ought to produce. No luck yet with preparation of material for electron microscope.

Since writing the above I have abstracted some pedigrees down on to this size of paper, and I will enclose them; I hope you can follow them. The one starting (1a) is the one which first put me on to the idea of E and T cells, the rest are more recent. The red figures in brackets are the minimum number of motile cells seen in progeny of cell indicated, that is minimum number of gene-products in the cell if it was a T cell. There are several limitations in these pedigrees; one is that there is a certain amount of mortality, even if cells not allowed to get out of log phase; another is that the probability of detecting a motile cell in the progeny of a T cell with 1 gene-product is evidently substantially less than 1, on some occasions anyway. See for instance pedigree starting with cell 3a, where of 18 motile cells (most of which must have been T cells with 1 gene product) 15 gave no detectable motile progeny. I think this is mostly a matter of failure to detect cell with flagellum, probably because it has got stuck, but loss of gene-product or death of cell bearing it could also account for it.

I do not share your scepticism (or caution) about interpretation of macro-trails. There may or may not be negative chemotaxis, but I don't see that its occurrence affects the evidence for existence of E cell and "phenotypic lag". As to the latter I have one additional observation; a motilised cell was picked to droplet, and transferred to gelatin-agar when it had formed 3 cells. These produced 1 single colony, and two pairs of twin-colonies, the numbers of a pair being about 1 mm. apart (centre to centre).

The only remaining theoretical difficulty is why there are such a much greater number of "initials" producing only a few motile progeny, that is why so many apparent T cells in relation to number of E cells? (1) Owing to my use of log-phase cells there are probably some cell divisions intervening between time phage applied and time motile cell trapped; if gene products, e.g. flagella anlage,

are distributed during these divisions, even though motility has not yet developed owing to phenotypic lag, one would expect some excess of T cells. However, I doubt if this will explain it all. Other possibilities would be (ii) transfer by phage of gene-products; this I reject for economy of hypothesis. Or (iii) there may be 3 alternative fates for phage-imported genes, (a) chromosomal incorporation in continuity to give transformation, (b) incorporation as side-arm or other odd position so that gene is not lost but is not replicated, giving E cell, or (c) loss after short delay e.g. at first cell division, delay being sufficient for gene to generate several gene products. Or (iv) the early E cell may be specially liable to die: this I think unlikely, as we have both pedigrees in which all progeny at 4 or 8 cell stage give clones.

In my material most of the macro-trails are fairly short, and do not extend on longer incubation. Similarly, I have failed in search for an E cell in progeny of earlier E cell in some micro-experiments though this does not mean much as it might have been stuck. Anyway I don't see any discrepancy now between micro and macro-trails.

I have now been able to get the same result, on two occasions, as you did in pedigrees giving motile clones, i.e. Sib of cell giving motile clone gave non-motile clone with a few motile cells. This phenomenon seems to me to fit in well with idea of quantal gene-product. In your letter of 1.3.54, page 2, you say (end of para) you have once had cells "corresponding to" 5-8th. generation giving swarm while othere gave semi-clones. Do you still interpret this as delay of "clonisation" to 5th. generation ? If so it is hard to fit in, but I am not sure if I have understood quite what you found. I have not found another example of macro trail becoming swarm. The one we saw was in SW 541 which I have never seen mutate so I don't think mutation will serve to explain it.

My attempt at mapping is now invalidated; on checking through the number of times I had tried the critical experiment, which was attempt to get "doubles" from SL 28 by treatment with phage grown on SW 543, I decided it was insufficient to be very sure about, so we tried several more times, using various lysates, and finally got doubles, in two replicate plates. That is, all the 6 interactions of SW543, SW 553 and SL 28 have now given "doubles" as well as "singles". I suppose the most likely explanation is what you call double crossing-over; I don't know if crossing-over is an appropriate term or not, if it is I should call incorporation of a single fragment a double, and of two fragments quadruple, crossing -over. All these interactions need re-investigating quantitatively: we may be able to infer something from the observed differences of the yields of doubles using Fla- and Fla+mutant lysates; but we have run into same trouble as you did I think, that is proportion of doubles varies from one experiment to another and some doubles come up late. I shall try to get back to this when trail business is cleared up.

In the course of the above experiments we have found that, as you said in your report, anti-i or anti-b serum inhibit trails from SW 543 treated with lysate of LT2, and we have checked that this is not due to cross-reacting antibody. My surprise at your statement was due, I find on checking my notes, to the fact that I had done the experiment only with H_1-linked strains other than SW 543, where the situation is different. I suppose it must mean all or nearly all E cells carry both H_1 genes; I now see why you expected to get recombinants from such E cells.

SW553

P.S. Quadling finds SL18 behaves like SW553. That is either serum inhibits trails.

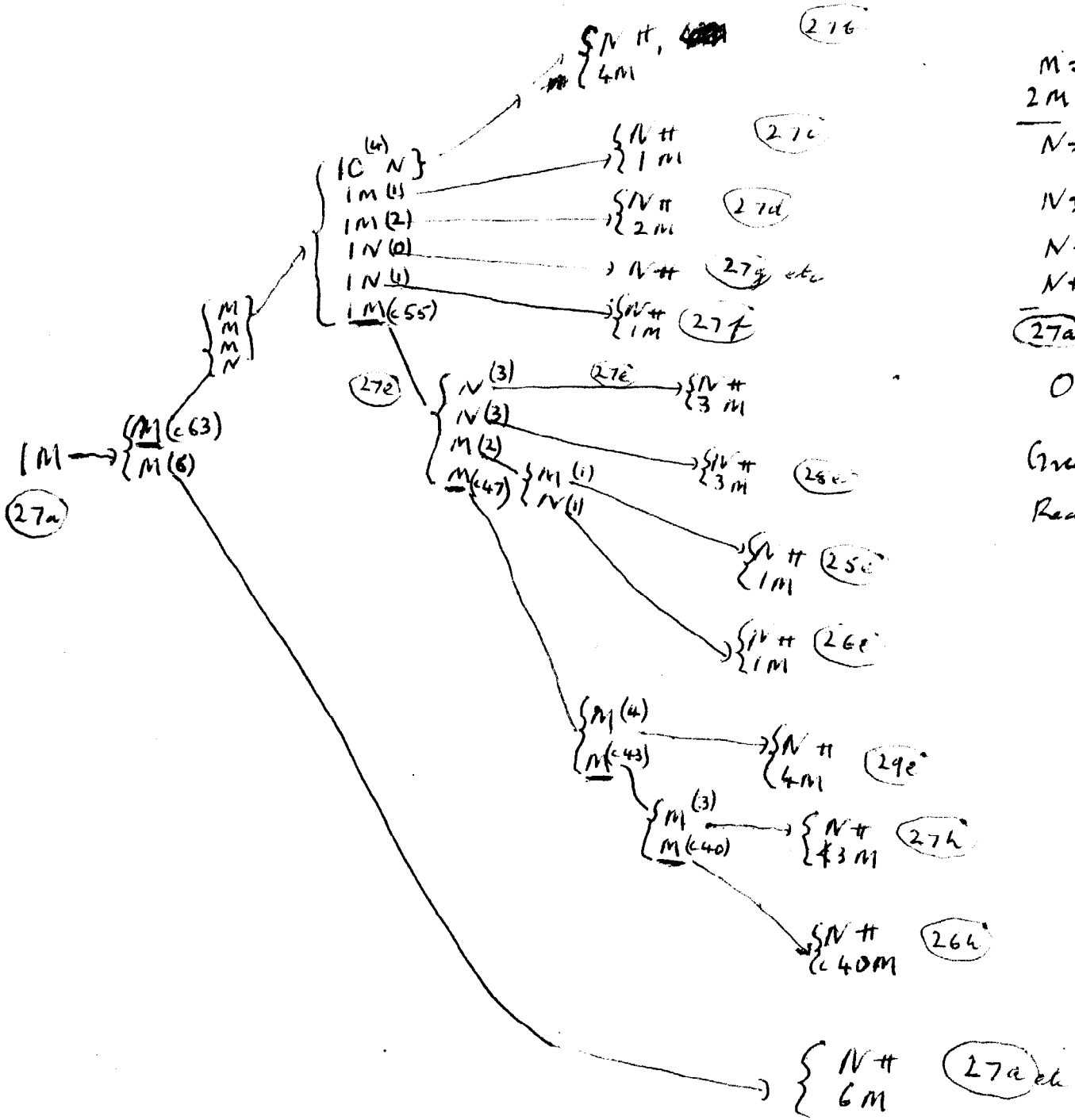
I wrote to Iseki and Sakai for re-prints of their last paper and they sent me copies (to N.Y.U.) marked for you and Edwards as well, I have sent yours on surface mail. What do you think of their stuff ? I feel pretty sceptical until someone repeats it. Freezing and thawing seems a strnge way to get a lysate.

Yours sincerely,

B.Stocker.

I congratulate you on your trapping of motile conjugant pairs. It sounds a pretty technique: have you tried O x O crosses at all ? However if there is much lag maybe you would have separation before motility developed. I was thinking of trying it when I got your letter. I hear Cavalli is going to spend some time with you. I had quite a pleasant week in Paris. Eggleston has some nice stuff on "suppressive littles", Hamnett Taylor was titrating transforming activity of DNA fractions from ? Rick. resin chromatography Aaron Novick was in London for a few days last week. My efforts to get pedigrees or any other kind of work done are being impeded by some sort of clamped-belly-ache which seems to be resistant to diagnosis, & has been a great nuisance recently. When are you & Esther coming for a trip to London? Pontecorvo was here a few days ago: someone working with him has an interesting result which is that if you get single crossing over between two of his pseudo-alleles you have a good chance of getting double crossing over within the very short section involved. Yrs Bruce

Don't forget another tape-letter ?



Key

M = motile cell

2M = 2 " " , etc

N = non-motile (or at least non-moving) cell.

N ± . hundreds of N cells.

N + thousands " " "

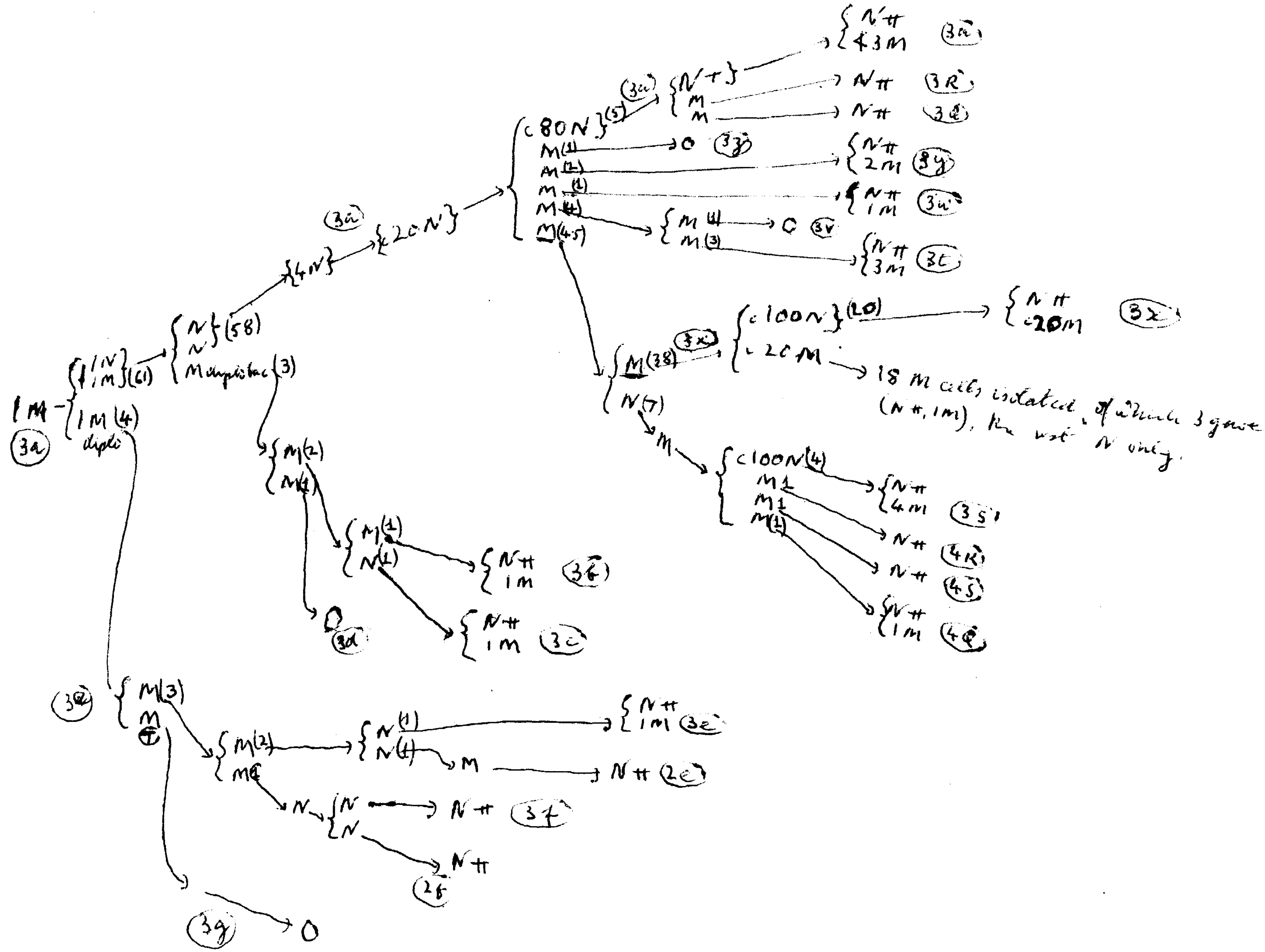
N ++ . many " " " "

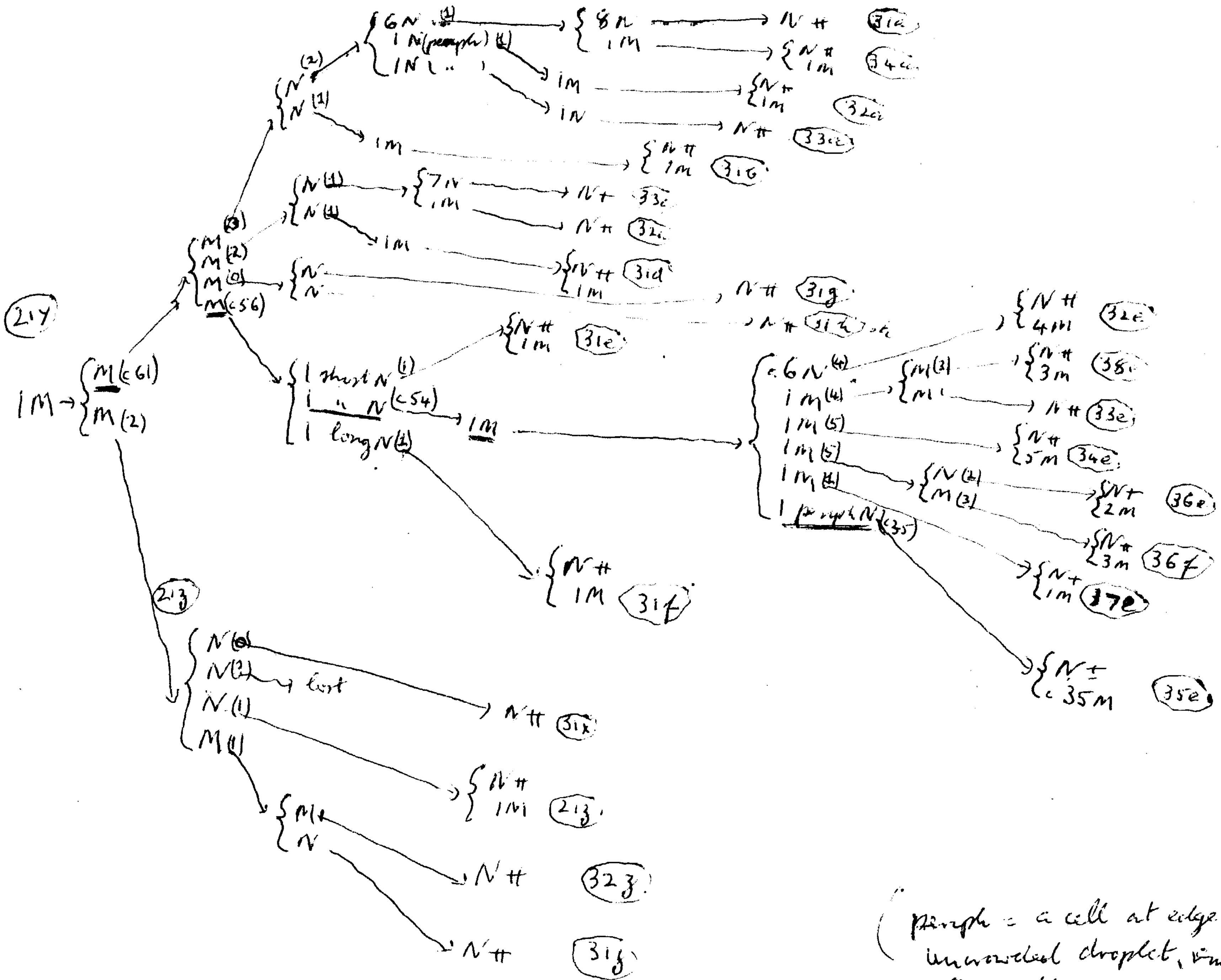
27a etc. Index of drop number of droplet.

O . Cell lysed, or failed to divide.

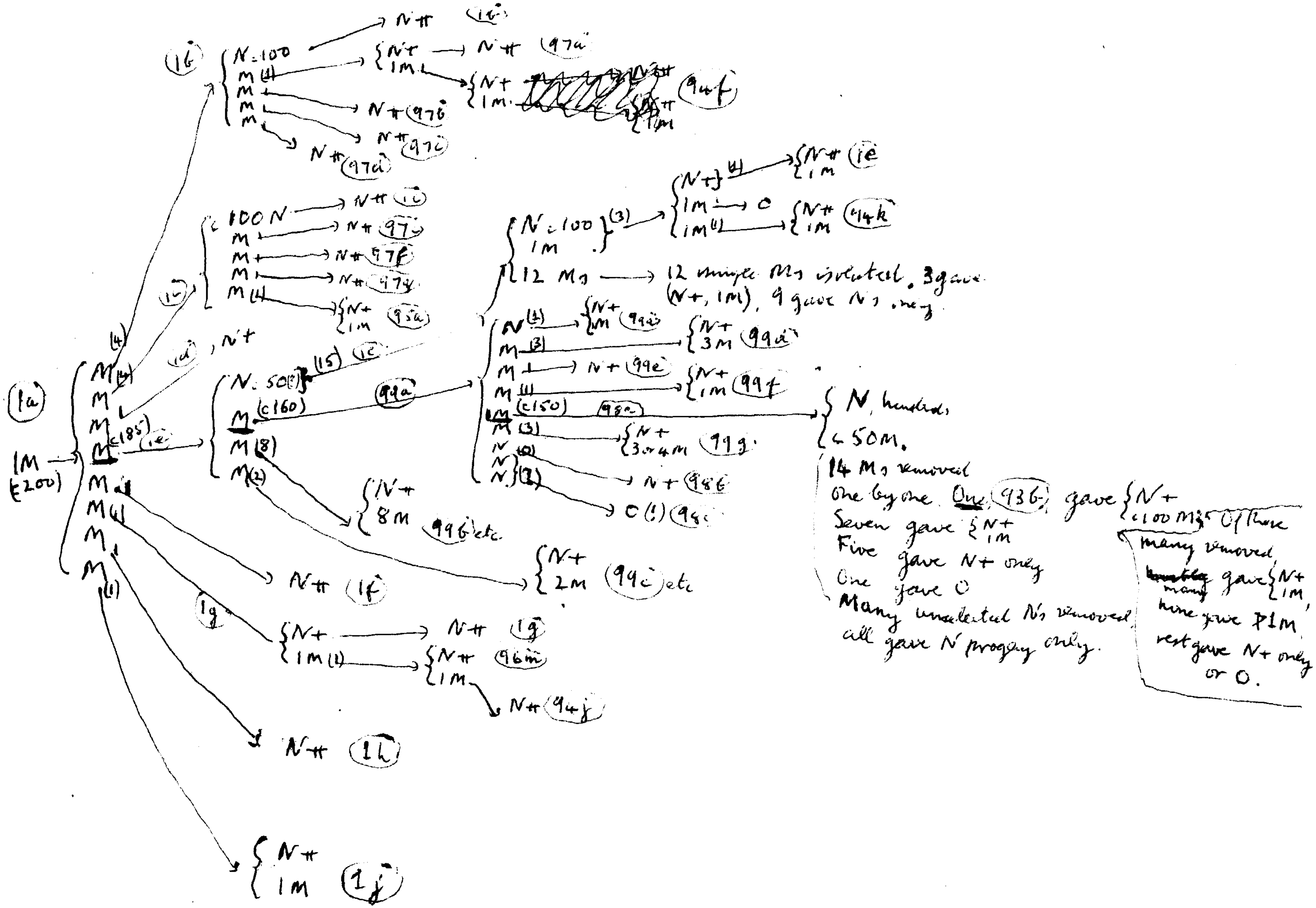
Green underlining . = E cell.

Red figures in brackets : total number of motile cells in progeny of cell (?) concerned.

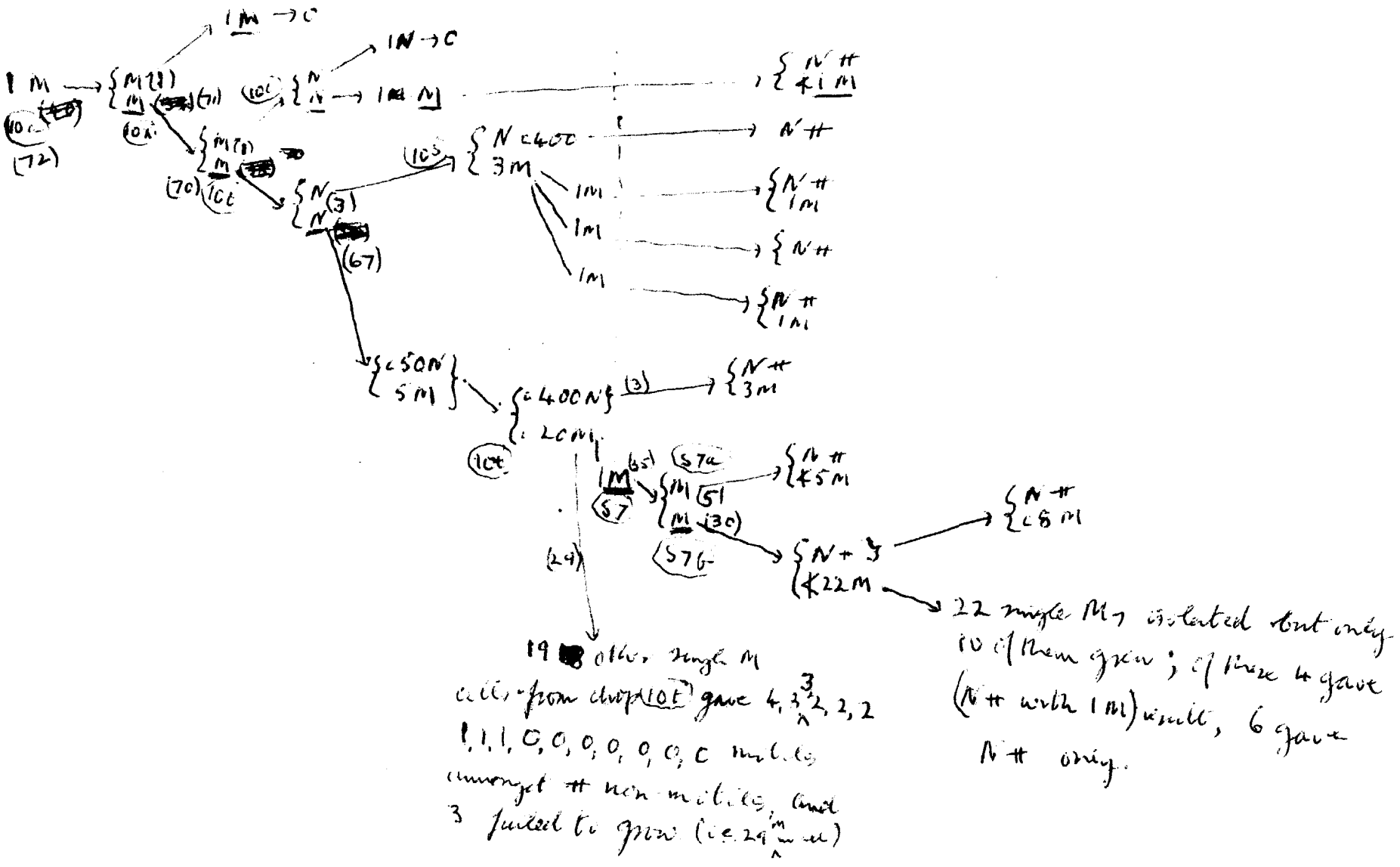

1M
3a
1N
1M (61)
1M (4)
diplo
N
N (58)
M
{4N}
{20N}
c80N (5)
M (45)
M (38)
N (7)
c100N (20)
c20M
18 M cells isolated, of which 3 gave (N+, 1M), the rest N only.
c100N (4)
N+
1M
3g
O



(periph = a cell at edge of uncrowded droplet, remote from other cells)



1a
1M (c200)
1b
N c100
N+ 1M
N+ 97a
N+ 1M 94f
N+ 97b
N+ 97c
N+ 97d
c100 N
N+ 97e
N+ 97f
N+ 97g
N+ 1m 95a
1d , N+
N c100 1M
12 Ms
12 single Ms isolated. 3 gave (N+, 1M), 9 gave Ns only
N+ 1M 1e
1M → O
N+ 1M 94k
N+ M 99b
N+ 3M 99d
N+ 99e
N+ 1M 99f
M (c150) 98a
N+ 3 or 4M 99g
N+ 98b
O (1) 98c
N, hundreds,
c 50M.
14 Ms removed
One by one. One (93b) gave N+ c100 Ms. Of these many removed, many gave N+ 1M, none gave >1M, rest gave N+ only or O.
Seven gave N+ 1M
Five gave N+ only
One gave O
Many unselected N's removed all gave N progeny only.
N 50
M (c160) 99a
M (8)
M (2)
N+ 8M 99b etc
N+ 2M 99c etc
M (c185) 1c
N+ 1f
1g
N+ 1M (1)
N+ 1g
N+ 1M 96m
N+ 94j
N+ 1h
N+ 1M 1j



C = cell failed to divide, or divided once only

(10c) etc. under number of previous drop

(10), etc. total number of M cells seen in progeny of cell

— green underlining = cell inferred to be gametocyte

M = motile cell

N = cell that non-motile cell (or not moving anyway)

N ++ = hundreds of N cells (estimated)

++ = thousands " " "

N ++ = tens of " " " "